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Multi-potentiality of a new immortalized epithelial stem cell line derived from human hair follicles

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Abstract

We previously demonstrated that keratin-15 expressing cells present in the bulge region of hair follicles exhibit properties of adult stem cells. We have now established and characterized an immortalized adult epithelial stem cell line derived from cells isolated from the human hair follicle bulge region. Telogen hair follicles from human skin were microdissected to obtain an enriched population of keratin-15 positive skin stem cells. By expressing human papillomavirus 16 E6/E7 genes in these stem cells, we have been able to culture the cells for >30 passages and maintain a stable phenotype after 12 months of continuous passage. The cell line was compared to primary stem cells for expression of stem cell specific proteins, for in vitro stem cell properties, and for their capacity to differentiate into different cell lineages. This new cell line, named Tel-E6E7 showed similar expression patterns to normal skin stem cells and maintained in vitro properties of stem cells. The cells can differentiate into epidermal, sebaceous gland and hair follicle lineages. Intact β-catenin dependent signaling which is known to control in vivo hair differentiation in rodents, is maintained in this cell line. The Tel-E6E7 cell line may provide the basis for valid, reproducible *in vitro* models for studies on stem cell lineage determination and differentiation.

Keywords

Stem cell; keratinocyte; hair follicle; multi-potent; skin

Introduction

Epithelial stem cells have been identified in the epidermis, hair follicle, and intestine as cells with a high *in vitro* proliferative potential and as slow-cycling label-retaining cells *in vivo* (Jones and Watt 1993; Barrandon and Green 1987; Lyle et al. 1998; Bach et al. 2000). Adult, tissue-specific stem cells are responsible for the regeneration of the tissues in which they reside during normal physiologic turnover as well as during times of stress (Slack 2000; Ito et al. 2005). Moreover, stem cells are generally considered to be multi-potent, possessing the capacity to give rise to multiple cell types within the tissue (Spradling et al. 2001). For example, rodent hair follicle stem cells can generate epidermis, sebaceous glands, and hair follicles (Taylor et al. 2000; Oshima et al. 2001; Morris et al. 2004).

Stem cells have become a valuable tool in biomedical research, due to their utility as an *in vitro* system for studying developmental biology, differentiation, tumorigenesis and for

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possible therapeutic utility. However, adult stem cells are difficult maintain in culture because once they are released from the *in vivo* stem cell niche, primary cells reach senescence after serial passage and months in culture, which results in the increased cell size, change in proliferative potential, and altered differentiation (Roh et al. 2005; Allsopp & Weismann 2002; Shanmuganathan et al. 2006). Therefore, the access to adult human stem cells for research has been somewhat limited. In an effort to relieve this problem and to study the properties of adult skin stem cells, we generated an immortalized skin stem cell line (Tel-E6E7) derived from human multi-potent epithelial stem cells and characterized the cells by comparing them with primary stem cells. We determined that Tel-E6E7 cells express stem cell markers such as keratin 15 (K15) (Lyle et al. 1998), β1 integrin and tenascin-C (Tumbar et al. 2004; Morris et al. 2004), and also possess similar stem cell characteristics as primary cells with respect to clonogenicity, migration, adhesion, and differentiation.

Materials and Methods

Isolation and culture of epithelial stem cells

Epithelial stem cells were isolated and cultured as described previously (Roh et al. 2004; 2005). Briefly, fresh adult human scalp skin from facelift procedures was collected with IRB approval. After the skin was treated with Dispase (Sigma) overnight at 4°C, hair follicles were plucked from the Dispase-treated skin, segregated into telogen club hairs based on their morphology under a dissecting microscope, and cut at the bulge region. Isolated tissue fragments were digested with 0.05% trypsin-EDTA (Gibco) for 10 minutes and then with a mixture of trypsin-EDTA and Versene (Gibco, 1.33:1) for additional 20 minutes at room temperature, and spun down for 5 minutes at 800 rpm. The supernatant was removed, and isolated cells were plated on mytomycin C (1.5 μg/ml DMEM for 2 hours, Sigma)-treated 3T3-J2 cells in keratinocyte medium (KCM) (Rheinwald and Green 1975; Roh et al. 2004) without EGF and then changed to EGF-containing KCM next day. Cells were grown at 37°C in a humid atmosphere containing 5% CO_2. Cells were fed with EGF-containing KCM every 2 days.

Establishment of HPV 16 E6/E7 immortalized cell line and clonal cultures

The primary cell line was immortalized by transduction with a retroviral vector (LXSN-16E6E7) packaged by the amphotropic fibroblast line PA317 (kindly provided by James Rheinwald, Harvard Medical School, Boston, MA). The primary cells (~350,000 cells) at passage 1 were plated on a feeder layer and cultured for 2 days. Then, the cells were co-cultured with mitomycin C-treated virus producing cells PA317/L(E6E7) SN (~200,000 cells) in KCM. After 6 days of co-culture, all 3T3-J2 and virus producing cells were removed by Versene, and mitomycin-treated 3T3-J2 NHP cells (neomycin, hygromycin, puromycin resistant, ~200,000 cells per well, kindly provided by James Rheinwald, Harvard Medical School, Boston, MA) were added, and cells were selected under 0.2 mg/ml of G418 (Gibco) for additional 6 days. For each passage, ~5000 cells were plated and cultured on a feeder layer in KCM. Cells were 50–60% confluent in a week and were split to next passage. For clonogenicity, ~2000 cells were cultured for 9–12 days, fixed with methanol, stained with 0.2% Crystal Violet (Fisher) in 2% methanol/water (v/v), and the images were scanned by using a scanner (Epson).

Co-culture of keratinocytes with DP cells

Dermal papilla tissue fragments were isolated from the skin by microdissection and used as explant cultures as described previously (Roh et al. 2004). Primary stem cells, Tel-E6E7 or HaCat cells were plated at a density of 5,000 cells/cm^2. Cells were allowed to attach overnight in KCM without EGF and then changed to fresh EGF-containing KCM along with 3T3-J2 inserts. After overnight incubation, half of the 3T3-J2 inserts were replaced with DP inserts and all cells were fed with fresh media. Transwell inserts (Corning) for 12-well plates were

prepared with 20,000 mytomycin C-treated 3T3-J2 cells per insert in KCM or 20,000 DP cells per insert in Chang medium C (Irvine scientific) supplemented with 10% FBS and P/S.

Sebocyte differentiation

Primary stem cells, Tel-E6E7 or HaCat cells were plated at a density of 20–25,000 cells/cm^2 and allowed to attach overnight in sebocyte media (DMEM and Ham F-12 (1:1, Gibco), 6% FBS (Gibco), 2% human serum (sigma), P/S, and EGF (10 nM, Sigma)] as describe prevbiouslty (Akimoto et al. 2005). Cells were induced for sebocyte differentiation with sebocyte differentiation media containing 10 nM insulin, 1 μM troglitazone (Cayman chemical, Ann Arbor, MI), 100 μM WY14643 (Sigma), and 10 nM dihydroxytestasterone (Sigma) in sebocyte media for 12 days. Cultured sebocytes were washed with propylene glycol twice for 5 minutes each, stained with 0.7% (w/v) Oil Red O (Sigma) in propylene glycol for 7 minutes with agitation, washed once with 85% propylene glycol in distilled water, and then rinsed in distilled water. Oil Red O staining was viewed with a light microscope.

Epidermal differentiation

Primary stem cells or Tel-E6E7 cells were plated at a density of 23,500 cells/cm^2 and allowed to grow in KCM containing EGF without the feeder layer. After 10 days, cells were visualized with a light microscope and subjected to Western blots for Keratin 1.

Adhesion assays

Tel-E6E7 cells and primary epithelial stem cells (~2000 cells each) were plated into collagen IV-treated wells and allowed to adhere for 0–10 minutes, 10–60 minutes and 60 min-overnight (16 hours), as previously described (Jones and Watt 1993; Roh et al. 2005). The culture dishes were pre-treated with 10 μg/ml human collagen IV (Sigma) in PBSABC (PBS supplemented with 1 mM $CaCl_2$ and 1 mM $MgCl_2$) overnight at 4°C and then blocked with 0.05 % BSA in water at 37°C for 30 minutes as previously described (Jensen et al. 1999). The cells were then fixed with methanol, stained with Crystal Violet and counted by using an inverted microscope.

Cell Migration

Tel-E6E7 cells were re-plated into a flask pre-treated with 50 μg/ml collagen IV as described (Roh et al. 2005). Cells were allowed to adhere for 10 minutes and then washed with KCM. A final concentration of 10 μM HEPES was added to the media and the flask was infused with 5% CO_2, sealed, and videotaped for 4 hours with 15 minutes between frames by time–lapse video microscopy on a heated stage to keep the culture temperature at 37°C. Three sets of migration experiments were done and all 103 cells were counted. Motility was measured from digitized films using a cell tracking extension (ICRF) written for IPLab (Scanalytics, Inc).

Immunofluorescence staining

Cells were harvested and plated on collagen-treated coverslips overnight. Cells were fixed with 10% formalin or acetone for 10 minutes, blocked for 1 hour in 5% goat serum in 0.1% Triton X-100/PBS, and stained with monoclonal anti-K15 (1:40 in 0.1% Triton X-100/PBS, Lab Vision, Fremont, CA) antibody or with monoclonal anti-β1 integrin (CD29, 1:100 in 0.1% Triton X-100/PBS, Immunotech, Westbrook, ME) antibody followed by FITC-conjugated anti-mouse antibody (1:100 and 1:200 in PBS, respectively, Dako). All coverslips were mounted with Vectashield (Vector) and imaged using a fluorescence microscope (Leica).

Cell staining for cytometry analysis

Cells were harvested and washed once with PBS. After a centrifugation at 800 rpm for 5 minutes, PBS was pipetted off and cells were resuspened in 50 μl of primary antibody solution

(10 μg/ml primary antibody in PBS) and incubated on ice for 60 minutes. After a PBS wash, cells were resuspended in 50 μl of rhodamine-conjugated secondary antibody solution (1:100 in PBS, Dako) for 30 minutes on ice in dark. After a PBS wash, cells were resuspended in 250 μl of PBS and analyzed by FACScan (Becton Dickinson). At least 5000 cells were analyzed for each sample. Rat and rabbit IgG were purchased from Sigma. The antibodies used were rat polyclonal anti-α6 integrin (G0H3) antibody from Immunotech and rabbit polyclonal anti-CD71 antibody from Santa Cruz.

Lef/Tcf reporter assay

Primary skin stem cells or Tel-E6E7 cells were plated at 40,000 cells/well in a 12-well plate in KCM without EGF. The cells were transfected with either the Lef/Tcf-TK or the pRL-TK (control vector) from Upstate Biotechnology, Inc. (Lake Placid, NY) with Effectene (Qiagen). After one day, the medium was changed to Wnt-3A conditioned media from Wnt-3A producing L-cells (ATCC). After 24hrs, the cells were lysed and luminescence was measured using the Dual-Luciferase reporter assay system (Promega). The relative absorbance units for Lef:Tcf reporter/control was calculated.

Results

Cell immortalization and culture

Adult epithelial stem cells isolated from human hair follicles in the telogen phase were immortalized at passage 1 by transduction with human papillomavirus 16/E6E7 and the resulting cell line was named Tel-E6E7. After transduction, immortalization was evaluated by serial passage of Tel-E6E7 cells for up to 30 passages in culture (Figure 1A). When cultured with a feeder layer of mitomycin-C treated 3T3 fibroblasts, Tel-E6E7 cells formed tight colonies consisting of small keratinocytes, similar to the phenotype of early passage primary telogen hair follicle stem cells (Figure 1B). Tel-E6E7 cells maintained a stable phenotype after 12 months of continuous passage, but tended to form tighter colonies than the primary cells and they required a longer time of trypsin incubation when harvesting. Additional experiments indicate that the Tel-E6E7 cells do not form anchorage independent colonies in soft agar assays, indicating that these immortalized cells do not possess the characteristics of cancerous cells.

Stem cell specific protein expression

It is widely accepted that keratin 15 (K15) and β1 integrin are highly expressed in epithelial stem cells from hair follicles (Jones and Watt 1993; Lyle et al. 1998; Tumbar et al. 2004; Morris et al. 2004). In order to verify whether Tel-E6E7 cells can be considered as the same type of cells to the primary stem cells, we compared the expression of epithelial stem cell markers. Immunofluorescence staining demonstrates that the Tel-E6E7 cell line expresses K15 and β1 integrin (Figure 2,Figure 3). By Western blot, both primary stem cells and Tel-E6E7 cells also express tenascin-C (Figure 2), which is upregulated in murine skin stem cells (Tumbar et al. 2004; Morris et al. 2004).

Since cells expressing high alpha6 integrin, but low CD71 are known to contain stem cell characteristics (Li et al. 1998), we also evaluated the expression levels of alpha 6 integrin and CD71 in Tel-E6E7 cells by using cell cytometry analysis. As demonstrated in Figure 3, 85% of Tel-E6E7 cells expressed alpha6 integrin, but only 1% were positive for CD71. These data suggest that Tel-E6E7 cells express multiple stem cell marker proteins and may be utilized like primary stem cells.

Multi-potentiality

One of the most important properties of stem cells is their capacity to differentiate into different cell lineages. Our results indicate that primary skin stem cells as well as the Tel-E6E7 cell line are able to differentiate into epidermal, hair follicle and sebaceous lineages. For *in vitro* hair differentiation, expression of Keratin 6 hair follicle (K6hf), which is specifically expressed in the companion layer of the hair follicle (Winter et al. 1998), increased after co-culture with dermal papilla cells for 2 days (Figure 4A). We previously showed that this induction of hair differentiation depended on active Wnt-β-catenin signaling (Roh et al. 2004). Using the Lef/Tcf reporter construct, we have determined that the Tel-E6E7 cells show a similar level of response to Wnt stimulation as primary stem cells (Figure 4). When primary stem cells or the Tel-E6E7 cells were cultured in media with high calcium concentration without the 3T3-J2 feeder layer, the cells exhibit epidermal-type differentiation with increased expression of keratin 1 (Figure 4B). Tel-E6E7 cells, like primary stem cells, can also be induced towards the sebocyte lineage. When cultured in sebocyte differentiation media, both primary cells and the Tel-E6E7 cell line show decreased expression of keratin 7, which is normally present in progenitor cells within the basal layer of sebaceous glands. In addition, the cells show Oil Red O positive globules in the cytoplasm consistent with differentiated sebocytes (Figure 5).

Unlike the skin stem cells and the new Tel-E6E7 cell line, HaCaT cells do not express the K6hf hair keratin and do not respond to dermal papilla-induced hair differentiation (data not shown). However, HaCaT cells can be induced toward sebaceous differentiation with decreased keratin 7 expression and presence of Oil Red O positive vacuoles (Figure 5).

Cell Migration

We and others have previously shown that the stem cells tend to migrate more slowly compared to the transit amplifying cells (Adams and Watt 1991; Roh et al. 2005). In order to characterize Tel-E6E7 cells, we examined the *in vitro* cell motility of the Tel-E6E7 cells. Cells were plated onto collagen-coated plates and allowed to adhere for 10 minutes, and cell migration was measured by time-lapse, video-microscopy. When compared the results to that of primary stem cells (0.102 +/− 0.008 microns/min, Roh et al. 2005), Tel-E6E7 cells were also relatively stationary; showing a migration rate of 0.103 +/− 0.0992 microns/min.

Cell Adhesion

As verified above, stem cells usually express high levels of adhesion molecules such as β1 and alpha6 integrins and tightly adhere to the basement membrane. Since stem cells are normally permanently adherent within the niche, Tel-E6E7 cells were examined for their ability bind to collagen IV. Both primary telogen stem cells and Tel-E6E7 cells were plated into wells pre-treated with collagen IV for the periods of 10 minutes, 60 minutes, and overnight (16 hour). Higher percentages of both primary stem cells and Tel-E6E7 cells attached to collagen IV within one hour; more than 90% of Tel-E6E7 cells and 75% of primary stem cells showed significant adherent property to collagen IV (Figure 6).

Discussion

In this study we have generated an immortalized cell line from primary adult epithelial stem cells isolated from the human hair follicle bulge region. We previously showed that keratinocytes of the hair follicle bulge region had properties of stem cells by both in vivo and in vitro analyses (Lyle et al. 1998; Roh et al. 2005). However, further study of skin stem cells has been hindered by the challenges of obtaining frequent fresh tissues and the time and effort required to expand primary cells, as well as their eventual senescence in vitro. Therefore, we have immortalized our primary epithelial stem cells and established a cell line that is not only

capable of extended proliferation, but also possesses similar markers and *in vitro* characteristics of their parental cells.

The immortalized humans skin stem cell line maintains the expression pattern of the primary cultured cells as well as the cells residing in the normal stem cell niche of the hair follicle bulge. We previously showed that the bulge region of the human hair follicle contains a population of slowly-cycling, label-retaining cells that express cytokeratin 15 and high levels of β1-integrin (Lyle, et al. 1998). Others have used alpha-6 integrin bright, CD71 dim as a marker of keratinocyte stem cells with extensive in vitro proliferative potential (Li et al. 1998). More recently, expression profiling of murine skin has identified a number of genes that are over-expressed in the stem cell compartment, including Tenascin-C (Tumbar et al. 2004; Morris et al. 2004). When placed in culture primary cells may show aberrant gene expression, however the Tel-E6E7 cell line maintains normal expression across a number of protein classes including the above cytoskeletal, cell surface and secreted proteins. Importantly, the cell line retains expression of keratin15. In neonatal mice, K15 and K14 are limited to the basal layers of epidermis and the outer root sheath of hair follicles (Lloyd et al. 1995), while in adult mice, K15 becomes much more restricted in the skin and localizes solely to the bulge (Liu et al. 2003). In mice, both K15 and K14 can pair with K5 in the skin and other stratified epithelia such as cornea, tongue and forestomach (Lloyd et al. 1995), however, keratin 15 forms fine, wispy fibers and not the large intermediate filaments typical of other keratins. Thus, keratin 15 appears to represent a primitive keratin and by its expression pattern seems to identify the undifferentiated progenitor cells of squamous epithelia. This tight restriction of keratin 15 suggests a cellular role not limited to structural integrity. Functional analyses of keratin 15, such as with knockout mice have yet not been reported, and thus the Tel-E6E7 cell line may allow for studies of the functional significance of K15 expression within stem cells.

The human Tel-E6E7 cell line exhibits the important quality of multi-potency for skin epithelial subtypes. In rodent skin, the cells in the hair follicle bulge region have been shown to regenerate all epithelial layers of the skin, including the epidermis, sebaceous gland and all cell types of the hair follicle (Oshima et al. 2001; Morris et al. 2004). Successful skin reconstitution with human bulge cells has not been reported, however cells from the human hair can regenerate an epidermis in raft cultures and be transplanted onto chronic wounds (Lenoir et al. 1993; Limat & Hunziker 2002). Although hair follicle cells and can repopulate the epidermis under wounding conditions, it appears that progeny of the bulge stem cells may not persist as long-term residents of the epidermis (Ito et al. 2005). When induced to differentiate with high calcium, both primary hair follicle stem cells and Tel-E6E7 cells show increased keratin 1 consistent with epidermal differentiation. We reported previously that primary stem cells from the human hair follicle can undergo hair differentiation when induced by the specialized inductive dermal papilla cells (Roh et al. 2004). Significantly, the Tel-E6E7 cell line maintains this capacity after immortalization and serial passage. This is in contrast to the epidermal-derived HaCaT cell line that does respond to the inductive signals of dermal papilla cells and does not exhibit hair differentiation. Interestingly, HaCaT cells do show the ability to differentiate towards sebocytes under appropriate conditions. HaCaT cells, primary cultured stem cells and the Tel-E6E7 cell line all show expression of keratin 7 which is expressed in the basal layer progenitors of the sebaceous gland (Bieniek et al. 2007), and when induced to differentiate there is decrease of keratin 7 and up-regulation Oil Red O positive lipid vacuoles characteristic of mature sebocytes. Thus, while HaCaT cells may be a good model for epidermal and even sebaceous differentiation, bulge-derived keratinocytes appear necessary for studies of hair differentiation.

Wnt-β-catenin-Lef/Tcf signaling is one of several important pathways involved in skin stem cell fate determination (Das Gupta & Fuchs 1999; Merrill et al. 2001; Nieman et al. 2002; 2003). We showed previously that nuclear accumulation of β-catenin and signaling through

the Lef-1 transcription factor was required for up-regulation of the hair specific keratin, K6hf (Roh et al. 2004), when keratinocyte stem cells are induced by dermal papilla cells. Since the Tel-E6E7 cells maintain a similar level of Wnt responsiveness on reporter assays and show features of hair differentiation, the cell line should prove useful for dissecting the complexities of Wnt-β-catenin-Lef/Tcf signaling in directing stem cell fate determination along different lineages. The cell line may also be a valuable resource for analyzing other signaling pathways involved in skin stem cell fate and hair biology, such as the hedgehog and BMP pathways.

In recent years, there has been an explosion of significant molecular and functional information describing the nature of hair follicle stem cells and their relationship to normal development, post-natal differentiation, fate determination, tumorigenesis and hair biology. However, much of the data relies on murine systems for molecular profiling and gene function studies while studies in human skin and hair has been much more limited. While not ignoring the power of the rodent systems, one must recognize the differences between mouse and human hair. Besides differences in size, structure, length of the hair cycle and response to androgens, it is evident through expression profiling that stem cells of the human hair follicle bulge differ molecularly from mouse hair follicle stem cells (for review, see Ohyama 2006). The translation of advances in mice to human skin and a better understanding of the differences may be enhanced by the availability of a stable human epithelial stem cell line that can be used with available in vitro assays of the stem cell phenotype.

In summary, immortalized multi-potent adult epithelial stem cells from human skin show a similar molecular and biologic phenotype to their parent primary cells and to the normal residents of the stem cell niche in the hair follicle bulge. The cell line appears stable for >30 passages and over 1 year of continuous culture. The cells differ from the epidermal-derived HaCaT cells in their ability to respond to induction of hair differentiation. Because transgenic and other approaches used to study hair biology in murine systems are not amenable to the study of human hair follicles, the Tel-E6E7 line may be a practical alternative as a renewal, clonal source of cells for study.

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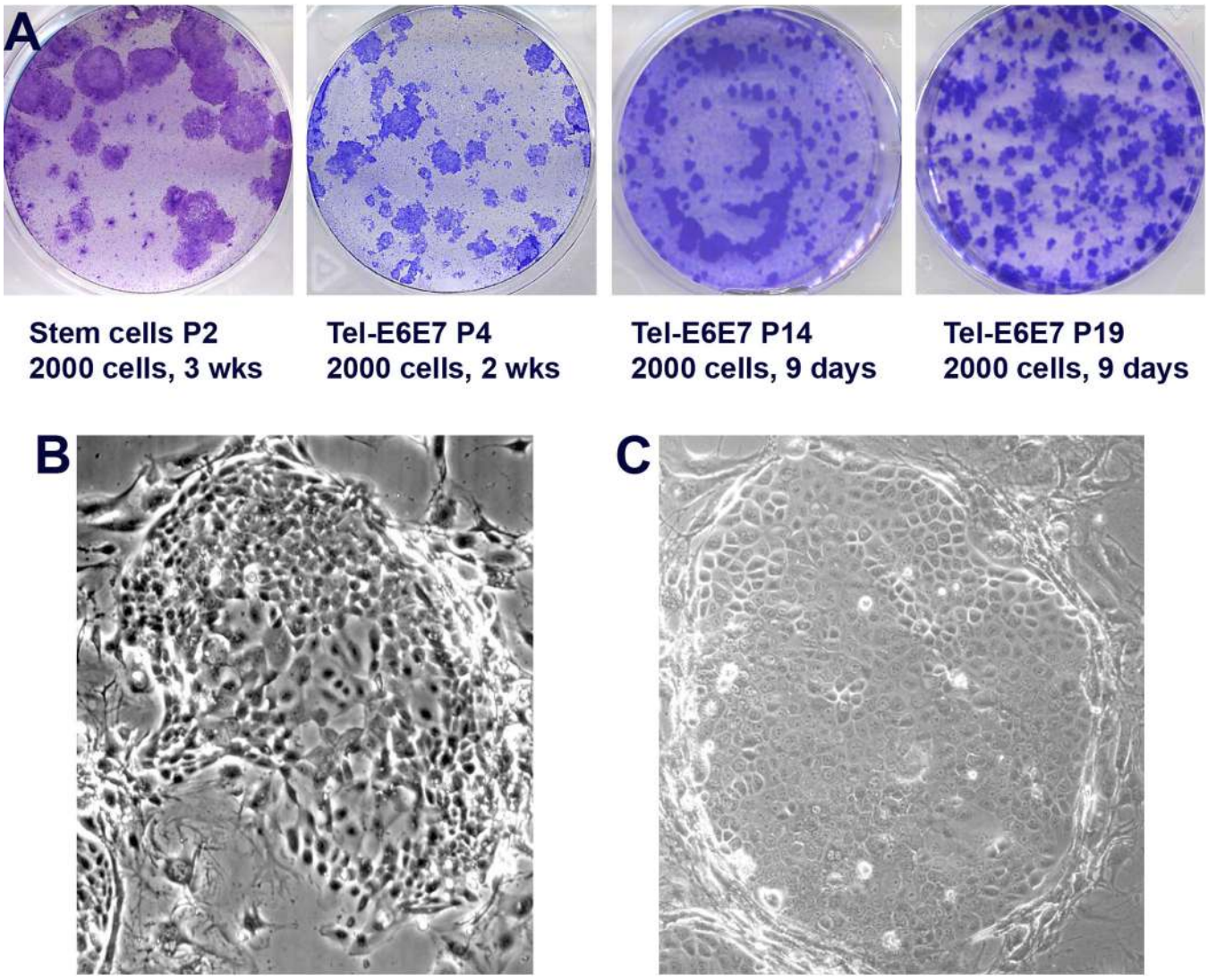

Figure 1.

Colony formation of the immortalized Tel-E6E7 cells. A. Cell staining of primary stem cells isolated from the human hair follicle bulge and the immortalized cell line (Tel-E6E7) at various passages. Cells were fixed with methanol and stained with crystal blue, and the images were scanned using a scanner. Tel-E6E7 cells were able to form colonies even after 8 months in culture. Primary stem cells as passage 3 (B) show a colony of keratinocytes with small peripheral cells and some larger more differentiated cells in the center. The immortalized Tel-E6E7 cells at passage 31 (C) shows a colony with uniform small keratinocytes. Original magnification 100X.

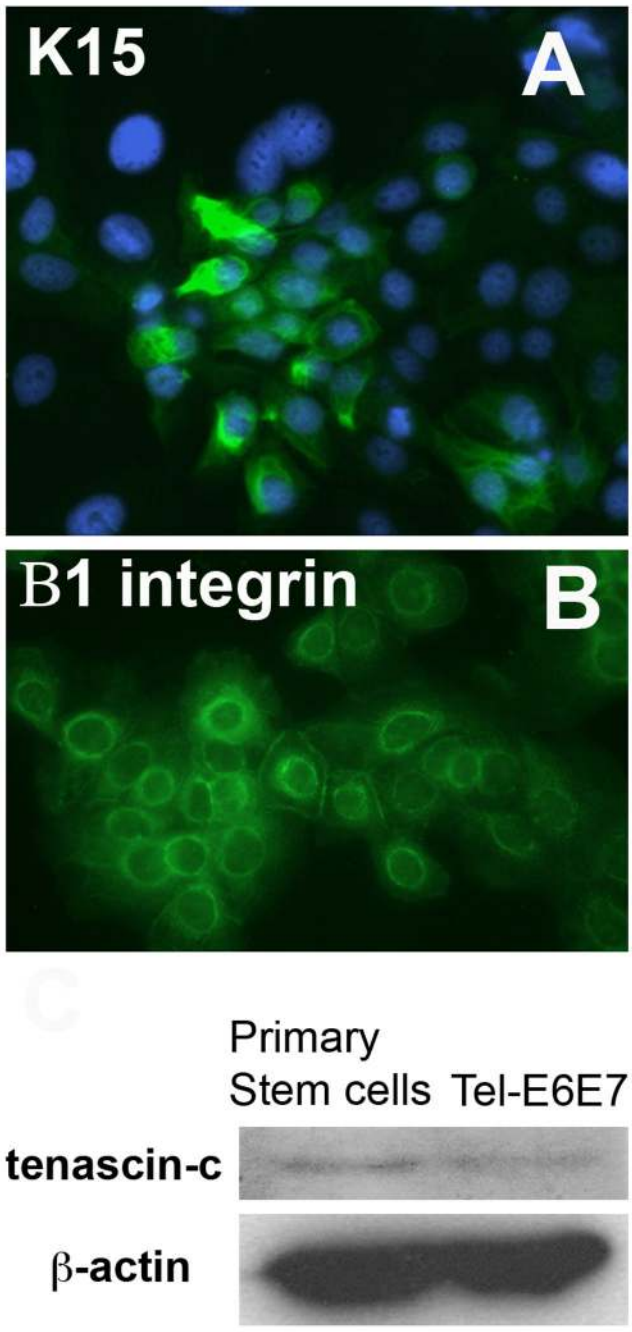

Figure 2.
Expression of epithelial stem cell markers in immortalized Tel-E6E7 cells. Tel-E6E7 cells positive cytoplasmic staining for K15 (A) and membrane staining for β1 integrin (B), similar to cells present in the hair follicle bulge. Both primary stem cells and Tel-E6E7 cells express the stem cell marker Tenascin-C.

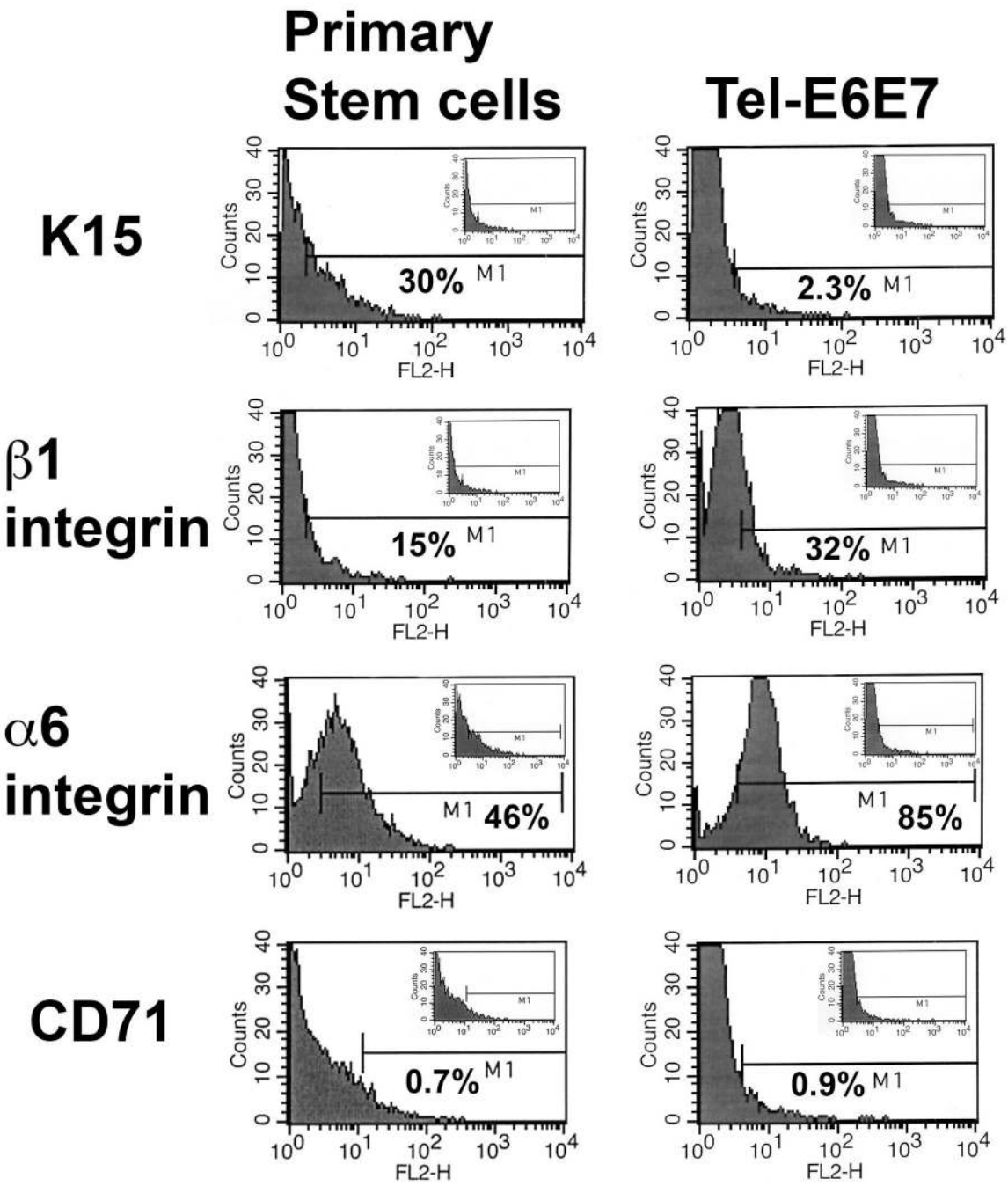

Figure 3.

Flow cytometric analysis of cultured primary stem cells and immortalized cells. Although the percentage of positive cells varies, the Tel-E6E7 maintains the same overall profile of K15, β1 integrin and α6 integrin expression and low expression of CD71. *Insets* - Isotype control traces for each antibody (K15 and β1 integrin - mouse IgG; α6 integrin - Rat IgG; CD71 - Rabbit IgG).

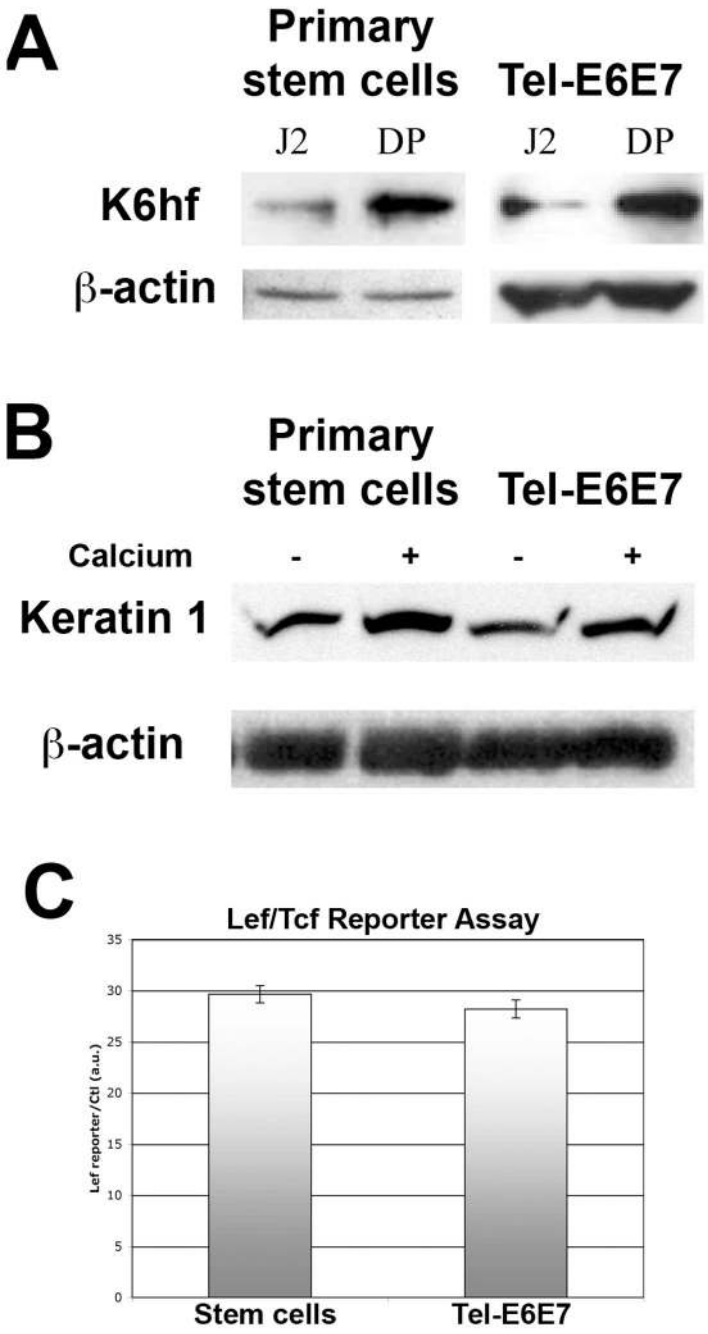

Figure 4.

Differentiation of Tel-E6E7 cells. A. Primary stem cells and Tel-E6E7 cells were induced by dermal papilla (DP) co-culture and show up-regulation of the hair follicle specific keratin K6hf compared to culturing with mouse fibroblast feeder cells (J2). B. Primary stem cells and Tel-E6E7 cells were cultured in high calcium media without a feeder layer and show increased keratin 1, indicative of epidermal differentiation. C. Primary stem cells and Tel-E6E7 cells were transfected with the Lef/Tcf reporter plasmid and then treated with conditioned media from Wnt-3A producing L-cells. Both cells show a similar level of Wnt-β-catenin-Lef/Tcf signaling in response to Wnt stimulation which is required for hair differentiation.

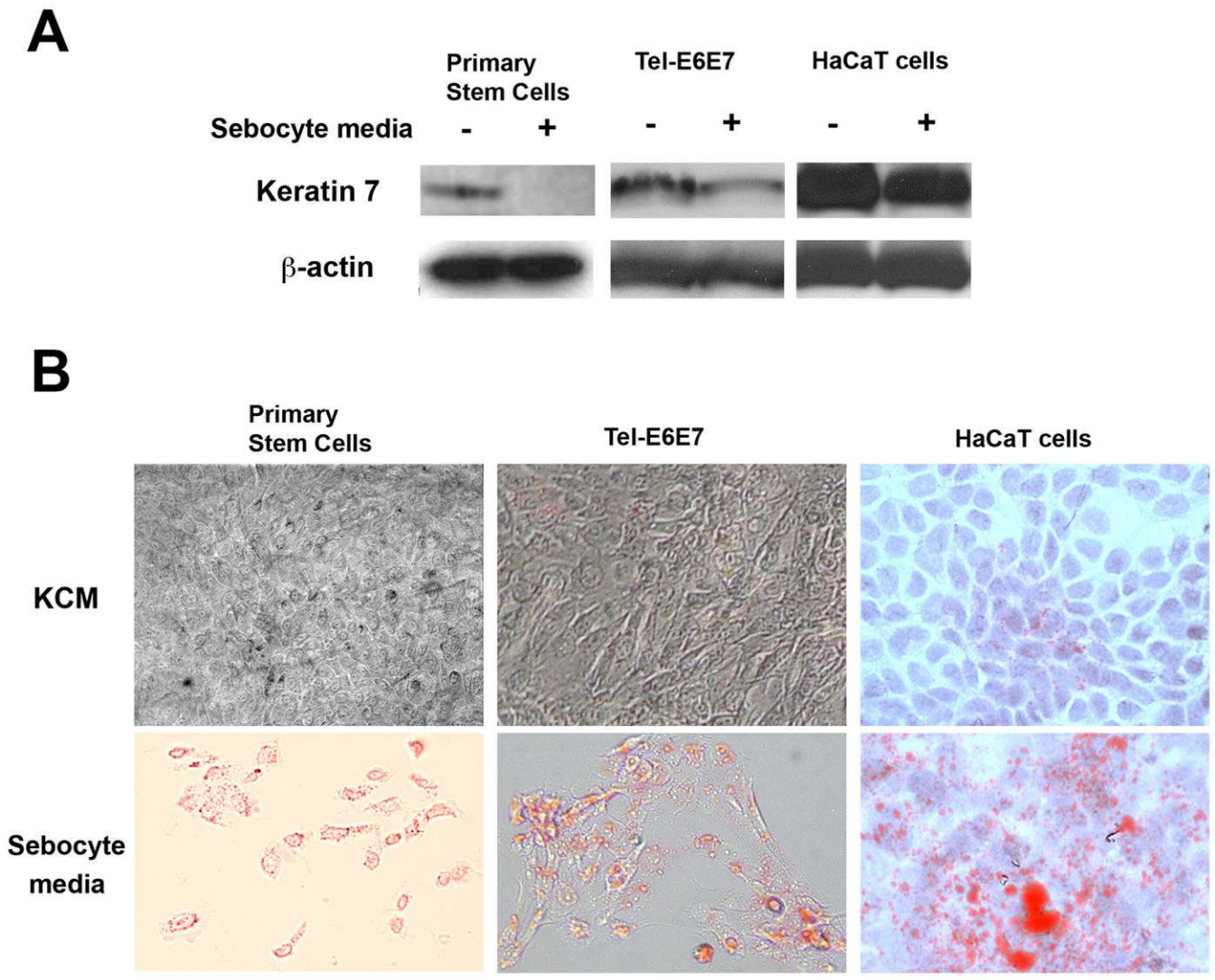

Figure 5.

Sebaceous differentiation of cell lines. Primary stem cells, Tel-E6E7 cells and HaCaT cells were treated with control keratinocyte media or sebocyte differentiation media. (A) Primary stem cells, Tel-E6E7 and HaCaT cells show a low level of keratin 7 expression which decreases with differentiation into mature sebocytes. (B) All three cells show increased Oil Red O positive lipid globules in the cytoplasm characteristic of mature sebocytes.

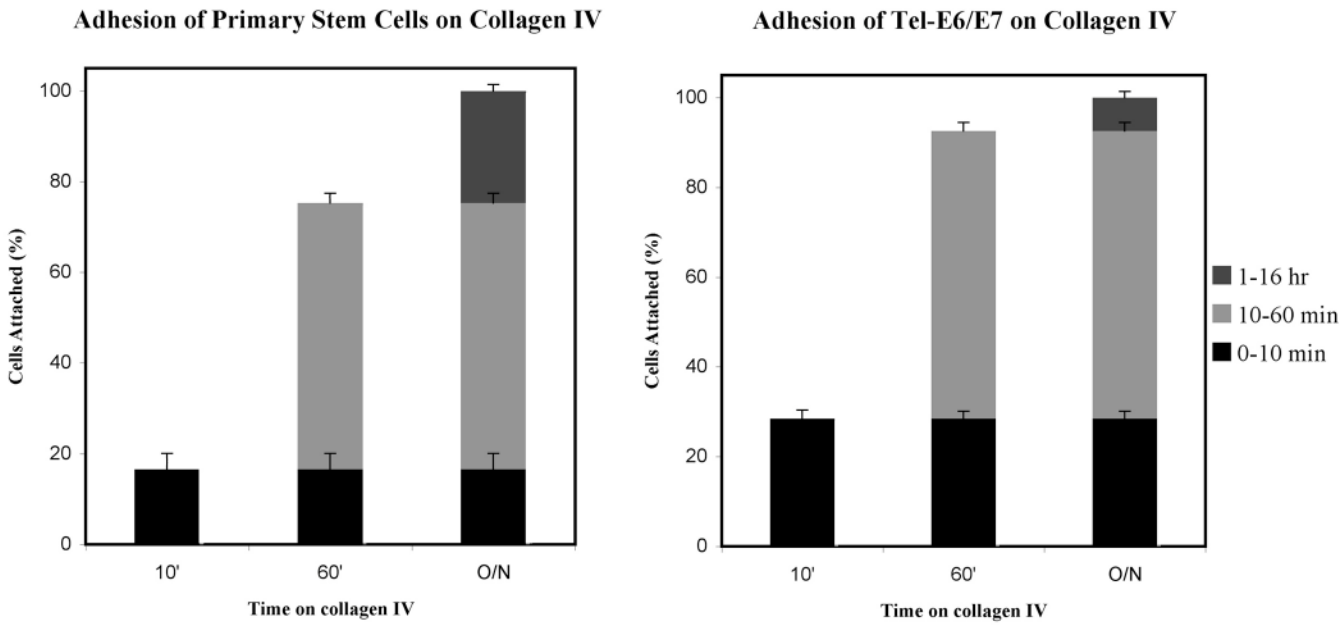

Figure 6.

Adhesion of Tel-E6E7 cells and primary stem cells. Both Tel-E6E7 cells and cultured primary stem cells were plated into collagen IV-treated wells for 10 minutes. Non-adherent cells were replated for 60 min., and then the remaining non-adherent cells were replated overnight (16 hrs). Cells were then fixed with methanol, stained with crystal violet and counted. The percentage of total adhering cells are plotted. The majority of bothTel-E6E7 and cultured primary stem cells attached to collagen-coated plates within 1 hour. Values show means +/− SE from 3 independent experiments.